RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER ACCORDING TO TUMOR SUBTYPES

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ABSTRACT

Objective: To compare the pathological response to neoadjuvant chemotherapy in different molecular subtypes of breast cancer.

Study Design: Prospective cohort study.

Place and Duration of Study: Department of Oncology Liaquat National Hospital Karachi from Jan 2013 to Dec 2014.

Material and Methods: A total of 119 patients received neo-adjuvant chemotherapy for locally advanced breast cancer followed by definitive surgery. Demographic, clinical and pathological data of 101 patients were available for analysis. Tumors were divided into different molecular subtypes, luminal A, luminal B human epidermal growth factor receptor 2 (HER 2) was negative, luminal B (HER 2 positive), HER 2 over expressed and triple negative. Neoadjuvant chemotherapy was given for total of eight cycles. Primary end point was pathological response [pathological complete response (PCR) versus no PCR] after surgery.

Results: A total of 101 patients data were analyzed. Seventeen (16.8%) were luminal A, thirty eight (37.6%) were luminal B, out of 38 luminal B patients, twenty one (55.2%) were HER 2 + and seventeen (44.7%) were HER 2 –ve. Sixteen (15.8%) patients were HER 2 over expressed and thirty (29.7%) were triple negative. Out of 101 patients, twenty eight (27.72%) achieved PCR. A total of 5.9% achieved PCR in luminal A, 4.8% had PCR in luminal B (HER 2 –ve type), 23.5% had in luminal B (HER 2 +ve type), 50% achieved PCR in HER-2 over expressed type and 46.7% had PCR in triple negative subtype, (p=0.001). There was no significant association of PCR with age, tumor size, lymph node status, histology or grade.

Conclusion: Molecular subtypes of breast cancer were found to be statistically significant predictor of PCR after neoadjuvant chemotherapy.

Keywords: Breast cancer, Chemotherapy, Molecular subtypes, Neoadjuvant, Response rate.

INTRODUCTION

Breast cancer is the most common cancer in women all over the world. Life time risk of being diagnosed with breast cancer is approximately one in eight female[1]. Over the past decade, neoadjuvant (pre-operative) chemotherapy has emerged as the standard of care in the treatment of inoperable and operable locally advanced breast cancer[2]. The aim of neo adjuvant chemotherapy is to downstage the tumor load to increase the rate of breast-conserving surgery and to gain information on in-vivo drug response. Patients who develop pathological complete response (PCR) after neoadjuvant chemotherapy have better clinical outcome in comparison to those who don't achieve PCR[3,4]. Gene expression profiling studies have shown that breast cancer is heterogeneous disease, consisting of subtypes with different molecular features and clinical outcomes[5]. Therefore, it is important to identify factors associated with the presence or absence of PCR after preoperative chemotherapy. Clinicopathologic characteristics such as clinical TNM stage (tumor, node, metastasis), age at diagnosis, estrogen receptor (ER) status, histological grade; have been associated with PCR after neoadjuvant chemotherapy[6,7]. Tumor with same clinicopathologic characteristics may be diverse in disease behavior, response to therapy and prognosis[8]. Studies have identified four major subtypes of breast cancer according to immunohistochemistry these are, (1) luminal A,

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Received: 11 Feb 2016; revised received: 24 Mar 2016; accepted: 28 Mar 2016

(ER+ve and/or PR+ve, Human Epidermal Growth Factor Receptor 2 (HER 2)-ve, Ki-67 <14 %,) (2) Luminal B, (ER+ve and/or PR+ve, HER 2-ve, Ki-67 >14%) OR, HER2+ve with any Ki-67) (3) Her2 over expressed (ER-ve, PR-ve, and HER2+ve) and (4) Triple negative (ER-ve, PR-ve, and HER2-ve)[9-12]. Recent meta analysis revealed significantly higher PCR rate to preoperative therapy among HER2+ve, and triple negative subtypes compared with luminal subtypes[13].

The prediction of the possibility of PCR[14,15] before starting neoadjuvant chemotherapy can be used to maximize the treatment and minimize unnecessary toxicity[16]. Purpose of this study is to assess the response of neoadjuvant chemotherapy in different subtypes of breast cancer in Pakistani population, so oncologist can plan neoadjuvant chemotherapy regimen according to breast cancer subtypes in future.

MATERIAL AND METHODS

This study was approved by the ethical and scientific committee of Liaquat National Hospital, Karachi.

This is a prospective cohort study and non-probability consecutive sampling technique was used. During two years, from Jan 2013 till Dec 2014, total of 119 patients received neo-adjuvant chemotherapy for locally advanced breast cancer followed by definitive surgery. Demographic, clinical and pathological data of 101 patients were available for final analysis. All patients were diagnosed by core needle biopsy.

Criteria for inclusion was, age ≥ 18 years, tumor greater than 5 cm or that involves the skin or chest wall or with fixed axillary lymph nodes.

Criteria for exclusion include patients with metastatic or recurrent or inflammatory tumor,

Table-I: Patients clinical characteristics.

	All (n=101)	Luminal A (n=17)	Luminal B [Her 2 Negative] (n=21)	Luminal B [Her 2 Positive] (n=17)	Her 2 over expressed (n=16)	Triple Negative (n=30)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Age (Years)						
≤ 35 years	18 (17.8)	1 (5.9)	1 (4.8)	4 (23.5)	2 (12.5)	10 (33.3)
>35 years	83 (82.2)	16 (94.1)	20 (95.2)	13 (76.5)	14 (87.5)	20 (66.7)
Clinical T						
cT3	41 (40.6)	11 (64.7)	6 (28.6)	5 (29.4)	6 (37.6)	13 (43.3)
cT4	60 (59.4)	6 (35.3)	15 (71.4)	12 (70.6)	10 (62.5)	17 (56.7)
Clinical N						
Negative	37 (36.6)	8 (47.1)	7 (33.3)	3 (17.6)	8 (50)	11 (36.7)
Positive	64 (63.4)	9 (52.9)	14 (66.7)	14 (82.4)	8 (50)	19 (63.3)
Histopathology						
IDC	89 (88.1)	16 (94.1)	18 (85.7)	15 (88.2)	15 (93.8)	25 (83.3)
Others	12 (11.9)	1 (5.9)	3 (14.3)	2 (11.8)	1 (6.2)	5 (16.7)
Tumor grade						
I	6 (5.9)	2 (11.8)	2 (9.5)	2 (11.8)	0	0
II	60 (59.4)	13 (76.5)	11 (52.4)	11 (64.7)	13 (81.3)	12 (40)
III	33 (32.7)	2 (11.8)	7 (33.3)	4 (23.5)	3 (18.8)	17 (56.7)
Unknown	2 (2)	0	1 (4.8)	0	0	1 (3.3)
Chemotherapy						
AC X 4	3 (3)	0	1 (4.8)	0	0	2 (6.7)
AC X 4 &Taxanes	77 (76.2)	17 (100)	20 (95.2)	5 (29.4)	7 (43.8)	28 (93.3)
AC X 4 & TH X 4	21 (20.8)	0	0	12 (70.6)	9 (56.2)	0

patients with bilateral carcinoma breast, and those who had previously received chemotherapy or hormonal therapy.

Initial tumour size and axillary lymph node status were assessed on clinical examination by measuring scale. Core needle biopsies were taken to determine the histological types, hormone receptor status, HER 2 Neu and Ki-67 status. Staging workup with computed tomography (CT) scan chest and bone scan was done to rule out metastasis. An over view of patients and their characteristics are given in table-I.

Total of eight cycles of chemotherapy was given. Neoadjuvant chemotherapy regimen, included adriamycin 60mg/m^2, with cyclophosphamide 600mg/m^2, over every 3 weeks, for total of 4 cycles, followed by docetaxel 100mg/m^2 over every 3 weeks, for another 4 cycles. Patients who were Her 2 Neu positive were offered trastuzumab according to their affordability. Three patients did not receive docetaxel.

The pathological response after neoadjuvant chemotherapy was taken as end point. Clinical response after every two cycles was assessed during neoadjuvant treatment. Patients who were showing good response, continued their treatment. Patients who showed disease progression during chemotherapy were referred for surgery.

ER/PR, scoring was done according to H-scoring system, a score above 10 was considered positive. The immuno-histochemistry (IHC) staining for HER 2 was scored according to standard criteria as 0, 1+, 2+, and 3+. Score of 0 and 1+ were considered negative and 3+ was taken as positive, on a score of 2+, additional FISH testing was done to establish HER 2 gene ampflication status. Staging (tumour, node, metastases) was done according to American Joint committee on Cancer (AJCC, 7th edition). PCR was defined as no residual invasive cancer in the excised tumor or lymph node, after neoadjuvant chemotherapy. Patients with residual carcinoma in situ (DCIS) were also considered as PCR.

Luminal A tumours were defined as ER+, PR+, HER 2 –ve, ki-67 ≤ 14%. Luminal B tumors were defined as ER+ve, PR+ve, HER 2 –ve, ki-67>14%, OR ER/PR+ with HER 2+ve. HER 2 over expressed tumor was defined as ER-ve/PR-ve and HER 2 +ve .Triple negative were defined as ER-ve/PR –ve and HER 2 –ve.

Primary end point was the pathological response (PCR vs. no PCR), according to molecular subtypes.

All data were analyzed with SPSS statistics software (version 22). Qualitative variables were computed by frequency and percentage and quantitative variables were presented by mean and standard deviation. For univariate analysis, Chi-square test or Fisher exact test was used to assess the relationship between the different subtypes and odd ratio with 95% confidence interval was computed. In univariate analysis variables, whose *p*-value was ≤20 were included in multivariate logistic regression model. A *p*-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics and distribution of molecular subtypes are reported in table-I. A total of 119 patients received neoadjuvant chemotherapy during 2 year period from Jan 2013 till Dec 2014.

Six patients develop clinical disease progression during chemotherapy; one patient expired during treatment, ten patients lost to follow-up during and after neoadjuvant treatment. Details of pathology of one patient was not available.

Data of 101 patients were available for final analysis. The mean age was 44.56 ± 10.04 years. Eighteen (17.8%) were <35 years of age, and 83 (82.2%) were >35 years of age. Seventeen (16.8%) patients were luminal A, thirty eight (37.6%) were luminal B, out of 38 luminal B patients, twenty one (55.3%) were HER 2 +, and seventeen (44.7%) were HER 2-ve. Sixteen (15.8%) patients were

HER 2 over expressed and thirty (29.7%) were triple negative.

Forty one (40.6%) patients had cT 3 tumor, and sixty (59.4%) had cT4. Whereas thirty seven (36.6%) had clinical axillary node negative disease and sixty four (63.4%) node positive disease. Pathology of eighty nine (88.1%) patients were infiltrating ductal carcinoma. Sixty (59.4%) tumors were grade II, thirty three (32.7%) were grade III and six (5.9%) were grade I, whereas grades of 2 patients were not available.

Seventy seven (76.2%) patients received regimen containing adriamycin, cyclophosphamide followed by docetaxel, whereas three patients did not receive docetaxel.

Twenty one patients received trastuzumab with above regimen. Out of these, twelve were luminal B HER 2 positive and nine were her 2 over expressed. Whereas twelve her 2 positive patients could not get trastuzumab because of financial issues.

Of 101 patients analyzed 28 (27.72%) patients achieved PCR, and 73 (72.27%) patients did not achieve complete response, the rate of PCR differed significantly among 4 molecular subtypes. About 5.9% (1/17) achieved PCR in luminal A, 4.8% (1/21) had PCR in luminal B (HER 2 –ve type), 23.5% (4/17) in luminal B (HER 2 +ve type), 50% (8/16) achieved PCR in HER-2

Table-II: Univariate analysis factors associated with PCR.

Variables	n	Pathological Response		*p*-value	RR [95%CI]
		PCR (n=28)	Non-PCR (n=73)		
		n (%)	n (%)		
Age (Years)				0.09*	
≤ 35 years	18	8 (44.4)	10 (55.6)		2.08 [0.92-4.71]
>35 years	83	20 (24.1)	63 (75.9)		Ref
Clinical T					
cT3	40	13 (32.5)	27 (67.5)	0.38	1.47 [0.76-2.06]
cT4	61	15 (24.6)	46 (75.4)		Ref
Clinical N				0.42	
Negative	37	12 (32.4)	25 (67.6)		1.25 [0.73-2.13]
Positive	64	16 (25)	48 (75)		Ref
Histology					
IDC	89	24 (27)	65 (73)	0.73*	0.96 [0.81-1.14]
Other	12	4 (33.3)	8 (66.7)		Ref
Grade**					
I & II	66	16 (26.7)	50 (75.8)	0.21	0.81 [0.57-1.16]
III	33	12 (36.4)	21 (63.6)		Ref
Chemo					
ACx4 &Taxanes	79	21 (27.6)	59 (73.7)	0.62	0.94 [0.74-1.20]
ACx4 & THx4	21	7 (31.8)	14 (19.2)		Ref
Molecular subtype					
Luminal A	17	1 (5.9)	16 (94.1)		Ref
Luminal B (Her 2	21	1 (4.8)	20 (95.2)		0.13 [0.02-0.92]
Negative)	17	4 (23.5)	13 (76.5)		0.80 [0.28-2.25]
Luminal B (Her 2	16	8 (50)	8 (50)	0.001*	2.60 [1.08-6.27]
Positive)	30	14 (46.7)	16 (53.3)		2.28 [1.29-4.03]
HER2~overexpressed					
Triple Negative					

** 2 patients showed unknown grade

* Fisher Exact test used

over expressed type and 46.7% (14/30) had PCR in triple negative subtype.

In univariate analysis, only molecular subtype were found to be statistically significant predictor of PCR (p=0.001). In the multivariate analysis (table-III), statistically significant association was seen with HER-2 over expressed and triple negative subtypes (p=0.005, p=0.007 respectively). There was no significant association of PCR with age, tumor size, lymph node status, histology, or grade.

When data were further analyzed according to patients who received trastuzumab, there was no significant benefit of addition of trastuzumab that might be due to small sample size (table-II).

Table-III: Multivariate stepwise logistic regression analysis; factors associated with PCR.

Factors	Sig.	Relative Risk	95% CI for odd ratio	
			Lower	Upper
Molecular subtype Luminal A		Ref		
Luminal B (Her 2 Negative)	0.90	0.13	0.02	0.92
Luminal B (Her 2 Positive)	0.17	0.80	0.28	2.25
HER2~overexpressed	0.016*	2.60	1.08	6.27
Triple Negative	0.014*	2.28	1.29	4.03

Model Accuracy = 71.7% Nagelkerke R Square = 0.28
Dependent variable= PCR independent variables = Molecular subtype, age and grade
Age and grade excluded from the model due to insignificant association.
Other variables were not included in the model because in univariate, p-value was >0.20.
*significant

DISCUSSION

Breast cancer is regarded as heterogeneous disease. Heterogeneity has been confirmed by gene expression profiling, that revealed various intrinsic breast cancer subtypes. The intrinsic subtypes based on gene expression analysis, first defined by Sorlie et al. in 2001[17]. The classification of breast cancer into subtypes on the basis of gene expression profiling is often regarded as the gold standard, but its widespread use either in clinical or in research settings remains limited due to the cost and technical difficulties. Consequently there is interest in using immunohistochemical (IHC) markers to classify tumours into subtypes that are surrogates for those based on gene-expression profiling. Simple IHC/FISH based method (Lips et al 2013)[18] also offer a prediction of response to neoadjuvant chemotherapy. In current study, breast cancer is divided into four molecular subtypes according to 2011 St Gallen consensus; luminal A, luminal B, Her 2 over expressed and triple negative. Luminal B is further subdivided into Her 2 positive, and HER 2 negative types. In Pakistan, Khokhar et al (2013) investigated association between clinical response to chemo-therapy and different molecular subtypes of breast cancer in Pakistan. In this study, it was investigated that if different molecular subtypes could help to predict neoadjuvant chemotherapy benefit in term of pathological response in breast cancer patients in Pakistan.

In a meta-analysis by Houssami et al (2012), an independent association between breast cancer subtypes and pathological complete response was established. It showed highest association with triple negative and HER 2 Neu over expressed subtypes and being more beneficial if anti Her 2 therapy was incorporated in latter type.

There is no association of pathological response with initial clinical stage, grade, and age in current study. This is in contrast to Gepar Trio study (Houber et al, 2010), where there was significant association of response with age, stage and high grade especially in triple negative subtype.

As we know from results of NSABP B-27 that addition of taxanes doubles the rate of

response. In this study all patients (except four) were given taxanes, so can't comment on association of chemotherapy regimen with response rate as there is no control arm. Similarly out of 33 HER 2 positive patients 21 patients received Trastuzumab, while 12 patients couldn't receive due to financial issues. Because of small number in these groups, addition of trastuzumab is not statistically significant. This is in contrast to established results, as from Gepar Quattro Study 2010, and TECHNO Trial 2011, that showed improved response rate with addition of trastuzumab in chemotherapy.

In current study, PCR rate is slightly high as documented in literature (meta-analysis by N Houssami et al, 2012), that might be explained by low proportion of luminal A in study population.

Distribution of PCR in different subtypes has a slight difference as reported in literature. In a study conducted by Minho et al 2011, there was no PCR in luminal A, while response in triple negative group was comparable with this study and PCR rate was around 30% in her 2 over expressed. While in current study there is 50% PCR in this group, this can be attributed to not incorporating trastuzumab in their regimen, while in current study 21 out of 33 Her 2 positive patients got trastuzumab.

In meta-analysis by Houssami et al (2012) response rate of 38.9% in Her 2 over expressed type was observed followed by triple negative (31.1%), very similar to this study.

These results can have important management implications in neoadjuvant settings that can be translated in adjuvant setting as well. As luminal A and luminal B HER 2 negative are less chemo sensitive, so chemotherapy toxicities can be avoided in these subtypes without compromising on response rate and prognosis (Minkwitz et al 2013)[19].

These results also emphasizing that luminal B her 2 positive and luminal B her negative are two different entities with respect to their biological behavior.

This study has limitations because of small sample size and low power. There is need to conduct local trials on larger scale.

CONCLUSION

Different molecular subtypes were found to be statistically significant predictor of pathological response in breast cancer after neoadjuvant chemotherapy. Biology of breast cancer is having a major role in deciding the course of disease and response to systemic therapy. These findings would have an important consideration in treatment decision in future.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. American cancer society, Atlanta, Georgia, Breast cancer facts and figures 2015-2016, American cancer society.
2. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998; 16(8): 2672–85.
3. Esserman LJ, Berry DA, DeMichele A, Carey L, Davis SE, Buxton M, et al. Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: results from the I-SPY 1 TRIAL-CALGB 150007/150012, ACRIN 6657. JCO 2012; 30(26): 3242-49.
4. Kong X, Moran MS, Zhang N, Haffty B, Yang Q. Meta-analysis confirms achieving pathological complete response after neoadjuvant chemotherapy predicts favorable prognosis for breast cancer patients. Eur J Cancer 2011; 47(14); 2084-90.
5. Polyak K. "Heterogeneity in Breast cancer." J Clin Invest 2011; 121(10); 3786-88.
6. Loibl S, von Minckwitz G, Untch M, Denkert C. Predictive factors for response to neoadjuvant therapy in breast cancer. Oncol Res Treat 2014; 37(10): 563-8.
7. Yu Y, Xiang H, He XM, Yang HJ, Zong XY. Predictive factors determining neoadjuvant chemotherapy outcomes in breast cancer-a single centre experience. Asian Pac J Cancer Prev 2013; 14(4): 2401-6.
8. Huober J, von Minckwitz G, Denkert C, Tesch H, Weiss E, Zahm DM et al. Effect of neoadjuvant anthracycline-taxane-based chemotherapy in different biological breast cancer phenotypes: Overall results from the GeparTrio study. Breast Cancer Res Treat 2010; 124(1): 133–40.
9. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006; 295(21): 2492-502.
10. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ, et al. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St-Gallen International Expert Consensus on the primary therapy of early breast cancer. Ann Oncol 2011; 22(8): 1739-1747.
11. Minhao Lv, Beibei Li, Yongfeng li, Xiaoyun Mao, Fan Yao, Feng Jin. Predictive role of molecular subtypes in response to

neoadjuvant chemotherapy in breast cancer patients in northeast china. Asian Pac J Cancer Prev 2011; 12(9): 2411-7.
12. Kim SI, Sohn J, Koo JS, Park SH, Park HS, Park BW. "Molecular Subtypes and Tumor Response to Neoadjuvant Chemotherapy in Patients with locally advanced breast cancer. Oncology 2010; 79(1): 324-30.
13. Guiu S, Michiels S, André F, Cortes J, Denkert C, Di Leo A, et al. Molecular subclasses of breast cancer: how do we define them? The IMPACT 2012 Working Group Statement. Ann Oncol 2012; 23(13): 2997-3006.
14. Houssami N, Macaskill P, Von Minckwitz G , Marinovich ML, Mamounas E. Metaanalysis of the association of breast cancer subtypes and complete pathologic response to neo adjuvant chemotherapy. Eur J Cancer 2012; 48(18): 3342-54.
15. Rouzier R, Perou C M, Symmans W F, Ibrahim N, Cristofanilli M, Anderson K, et al. Breast Cancer Molecular Subtypes Respond Differently to Preoperative Chemotherapy. Clin Cancer Res 2005; 11(16): 5678-85.
16. Von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. JCO 2012; 30(15): 1796-804.
17. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 2001; 98(19): 10869–74.
18. Lips EH, Mulder L, de Ronde JJ, Mandjes IAM, Koolen BB, Wessels LFA et al. "Breast cancer subtyping by immunohistochemistry and histological grade outperforms breast cancer intrinsic subtypes in predicting neoadjuvant chemotherapy response," Breast Cancer Research and Treatment 2013; 140(1): 63–71.
19. Von Minckwitz G, Fontanella C. Selecting the neoadjuvant treatment by molecular subtype: how to maximize the benefit?, Breast 2013; 22(2): 149-51.